

CLAIMS

1 1. A method for transferring a polynucleic acid
2 sequence from a donor vector to an acceptor vector wherein
3 said donor vector includes a first antibiotic resistance
4 functioning sequence and said acceptor vector includes a
5 second antibiotic resistance functioning sequence
6 comprising:

7 (a) digesting said donor vector and said acceptor
8 vector with restriction endonucleases, which digesting
9 releases said polynucleic acid from said donor vector and
10 restricts said acceptor vector such that said released
11 polynucleic acid and said restricted donor vector are
12 capable of ligation,

13 (b) combining the unpurified digestion products
14 including said released polynucleic acid and said
15 restricted acceptor vector into a ligation reaction
16 mixture,

17 (c) transforming host cells with said mixture of step
18 (b),

19 (d) introducing said host cells of step (c) onto
20 plates consisting of growth medium containing a second
21 antibiotic to which hosts cells containing said second
22 antibiotic resistance functioning sequence are resistant,

23 (e) growing distinct colonies of said host cells in
24 the presence of a compound that changes color in the
25 presence of the expression product of said first antibiotic
26 resistance functioning sequence, and

27 (f) collecting host cells including said polynucleic
28 acid contained in said acceptor vector from colonies that
29 grow on said plates from step (e) and that do not exhibit a
30 color change indicating the presence of said first
31 antibiotic resistance functioning sequence.

1 2. The method of claim 1 wherein said compound is
2 provided in said growth media.

1 3. The method of claim 1 wherein said compound is
2 provided by introducing said compound onto the surface of
3 said plate.

1 4. The method of Claim 1 wherein said growth medium
2 comprises said compound and a charged polymer gelling
3 agent which is capable of retarding the diffusion of said
4 compound and the product of the interaction of said
5 compound with the expression product of the first
6 antibiotic resistance functioning sequence.

1 5. The method of Claim 4 wherein said charged
2 polymer gelling agent is a polycationic polymer.

1 6. The method of Claim 4 wherein said charged
2 polymer gelling agent is a polyanionic polymer.

1 7. The method of Claim 5 wherein said polycationic
2 polymer is chitosan.

1 8. The method of Claim 5 wherein said compound
2 includes a negative charge.

1 9. The method of Claim 6 wherein said compound
2 includes a positive charge.

1 10. The method of claim 1 wherein said compound is a
2 chromogenic beta lactamase substrate.

1 11. The method of claim 10 wherein said compound is
2 nitrocefin.

1 12. The method of claim 1 wherein said acceptor
2 vector is capable of homologous recombination with nucleic
3 acid sequences encoding adenoviral genes to form a
4 replication incompetent adenoviral vector.

1 13. The method of claim 1 wherein said first
2 antibiotic resistance functioning sequence provides
3 resistance to an antibiotic selected from the group
4 consisting of β -lactam, macrolide, aminoglycoside,
5 tetracycline, polypeptide, polyene, and nitroimidazole
6 classes of antibiotics.

1 14. The method of claim 1 wherein said first
2 antibiotic resistance functioning sequence provides
3 resistance to ampicillin.

1 15. The method of claim 1 wherein said second
2 antibiotic resistance functioning sequence provides
3 resistance to zeocin.

1 16. The method of claim 1 wherein said restriction
2 endonucleases present in said unpurified digestion
3 products in step (b) are inactivated.

1 17. A method for transferring a first library of
2 unique polynucleic acid sequences included in a library of
3 donor vectors into a second library including each of said
4 unique polynucleic acid sequences in the form of an
5 acceptor vector, wherein said donor vectors include a first
6 antibiotic resistance functioning sequence and said
7 acceptor vectors include a second antibiotic resistance
8 functioning sequence, comprising:

9 (a) digesting each of said donor vectors of said
10 first library and said acceptor vectors with restriction
11 endonucleases, which digesting releases each of said
12 polynucleic acids from said donor vectors and restricts
13 said acceptor vector such that each of said released
14 polynucleic acids and said restricted donor vector are
15 capable of ligation,

16 (b) combining into distinct ligation reaction mixture
17 compartments of a third library, each of the unpurified
18 digestion product including each of said released
19 polynucleic acids of said library and said restricted
20 acceptor vector ,

21 (c) transferring each of said distinct ligation
22 reaction mixtures into each of a multiplicity of distinct
23 transformation compartments containing host cells and
24 growth medium containing said second antibiotic and
25 transforming said host cells,

26 (d) growing distinct colonies of said host cells in
27 each of said compartments in the presence of a compound
28 that changes color in the presence of the expression
29 product of said first antibiotic resistance functioning
30 sequence, and

31 (e) collecting host cells including said polynucleic
32 acids contained in said acceptor vectors from colonies that
33 grow in said compartments and that do not exhibit a color
34 change indicating the presence of said first antibiotic
35 resistance functioning sequence.

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1 18. The method of claim 1 wherein said polynucleic
2 acid sequence is part of a set or library of polynucleic
3 acid sequences that are individually cloned into donor
4 vectors.

1 19. The method of claim 1 wherein said method is part
2 of an automated or high-throughput process.

1 20. The method of claim 1 wherein said acceptor
2 vector is an expression vector.

1 21. The method of claim 1 wherein said acceptor
2 vector is a viral expression vector.

1 22. The method of claim 1 wherein said acceptor
2 vector is a retroviral vector.

1 23. The method of claim 1 wherein said acceptor
2 vector is an adenoviral vector.

1 24. The method of claim 23 wherein said adenoviral
2 vector is an adenoviral adapter vector which contains the
3 left ITR and part of the E2B region, and in which the E1
4 region has been exchanged for a mammalian promoter, a
5 polylinker sequence, and a polyadenylation signal.

1 25. The method of claim 24 wherein said adenoviral
2 vector is pIPspAdApt10/Zeo-lacZpart as shown in Figure 5.

1 26. An adenoviral vector as defined in claim 25.

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